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Oxidative stability of sunflower oils supplemented with kenaf seeds extract, roselle seeds extract and roselle extract, respectively under accelerated storage

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Abstract

With the concern of adverse effects of lipid oxidation on food deterioration and human health, the antioxidant activities of kenaf seed extracts (KSE), roselle seed extracts (RSE) and roselle extracts (RE) were evaluated by comparing its oxidative stability in refined, bleached & deodorised (RBD) sunflower oils with that of in synthetic antioxidant, BHA. Established methods such as peroxide values (PV), p-anisidine values (AV), TOTOX values, free fatty acids (FFA), iodine values (IV), total phenolic contents (TPC), conjugated dienes (CD) and conjugated triene (CT) were employed to assess the extent of oil deterioration. During 24 days storage, consensus was accomplished based on the results assessed by PV, TOTOX, CD, CT, IV and TPC at which the antioxidant activities of KSE, RSE and RE were better than BHA. Surprisingly, the results obtained by AV and FFA assays showed the reversed. Among the extracts, RSE exhibited the best antioxidant activities. These suggest that KSE, RSE and RE may be used as potential source of natural antioxidants in the application of food industry to prevent lipid oxidation.

Introduction

Lipid oxidation is one of the culprits of deterioration in fats and oils that leads to the development of off-flavours and unpleasant tastes which results in shelf life reduction (Sikwese and Duodu, 2007). Besides affecting the nutritional quality of the products, during this process, it may generates potential toxic compounds through the action of free radicals and reactive oxygen species (ROS) that are harmful on human health and is implicated in degenerative diseases such as cancer and early ageing (Krishnaiah *et al*., 2010). With the concern of the adverse effects of lipid oxidation on food deterioration and human health, there is a need to develop the exogenous antioxidants to prevent not only the presumed deleterious effects of free radical in the human body, but also the deterioration of fats and other constituents of food stuffs (Rozman and Jersèk, 2009).

The commercial antioxidants such as antioxidant supplements and synthetic antioxidants are widely available in the markets and the use of them has been widespread around the world. In food industry, the application of synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), may possess potential health risks corresponding to carcinogenesis (Krishnaiah *et al*., 2010) and are negatively perceived by consumers. As such, an immerse deal of interest in the use of natural antioxidants derived from plant extracts has been developed and is expected to rise tremendously in the foreseeable future.

Plant extract offers a unique range of applications for holistic health and wellness. Secondary plant metabolites such as phenolic compounds from plant sources are highly valuable for their therapeutic attributes as antioxidants (Sikwese and Duodu, 2007). Carnosic acid was proved to show strong protective effects against lipid oxidation of sunflower oil during accelerated storage (Zhang *et al*., 2010). Potato peels and sugar beet pulp extracts can stabilize both sunflower and soybean oils very effectively (Mohdaly *et al*., 2010).

In this study, roselle (*Hibiscus sadariffa* L.) calyxes, roselle seeds and kenaf (*Hibiscus cannabinus* L.) seeds are being studied as potential antioxidant agents due to their high antioxidant activities (Ghafar *et al*., 2010). Furthermore, there is a high potential for the commercialisation of these products due to the

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low value of roselle seeds and kenaf seeds that being discarded as by-products. According to Agricultural Department Negeri Terengganu, Malaysia, there are almost half of the velvety capsules containing the roselle seeds being discarded as by-products in the calyxes removal process (Halimatul *et al*., 2007). In addition, in Malaysia, kenaf has recently received an increasing interest as the government has the tendency to cultivate kenaf as the fourth industrial crop in the nation to replace tobacco plantation (Chan and Ismail, 2009). As such, concomitant with the increase availability of the raw materials due to large-scale cultivation and being discarded as industrial wastes, there are high potentials of commercial exploitation of these crops as potential antioxidants.

Hence, in the present studies, the oxidative stability of RBD sunflower oils supplemented with plant extracts derived from kenaf seeds, roselle seeds and roselle calyxes, respectively were being investigated and compared with synthetic antioxidant, BHA to justify their potential uses as natural antioxidants in various industries including food industries.

Materials and Methods

Plant materials

The dried calyxes of roselle (*Hibiscus sabdariffa* L.) were purchased from Hokuden (M) Sdn Bhd (Johor, Malaysia). Dried kenaf (*Hibiscus cannabinus* L.) and roselle (*Hibiscus sabdariffa* L.) seeds were obtained from Malaysian Agricultural Research and Development Institute (MARDI) (Selangor, Malaysia).

Samples preparation

Extraction

Each plant materials (kenaf [*Hibiscus cannabinus* L.] seeds, roselle (*Hibiscus sabdariffa* L.) seeds and roselle calyxes) was grounded into fined powder using grinder (EM-11, Sharp, Japan) to reduce the particles size respectively. The dried sample powders (500 g) were placed in a 2000 mL round-bottom flask with 1300 mL of deionised water, respectively in order to carry out hydrodistillation for 4 hours or until there was no more essential oil yield. After 4 hours, the liquid retentate from each hydrodistillation were centrifuged at 4500 rpm for 20 minutes and the supernatant was filtered using filter paper (Whatman No. 1, Whatman International, England) to obtain a clear solution of water extract. The collected filtrates were then undergone rotary evaporation at 45°C using rotary evaporator (Rotavapor R-200, BUCHI Labortechnik AG, Switzerland) until sticky extracts were obtained. The sticky extracts obtained were oven dried at 65°C for 1 day to remove excessive water. It was then kept at -20°C prior to further use.

Accelerated storage

Kenaf (*Hibiscus cannabinus* L.) seeds extract, roselle (*Hibiscus sabdariffa* L.) seeds extract and roselle extract (1500 ppm each) were weighed and dissolved in 5 mL of 2-propanol to facilitate dispersion (Sikwese and Duodu, 2007) before mixed with 150 mL of refined, bleached & deodorised (RBD) sunflower oil, respectively in a 250 mL Scott bottle, which was wrapped with aluminium foil. Fresh RBD sunflower oil (150 ml) was used as control while legal limit of 200 ppm of butylated hydroxyanisole (BHA) was weighed and dissolved in 5 mL of 2-propanol before mixed with 150 mL of RBD sunflower oil was used as standard in this study. All oil samples were prepared in duplicate. The oils were then stored in an oven (1350 FX, SheL Lab, USA) at 65 °C for 24 days to accelerate the deterioration of the oil. Approximately 30 mL of the oils were withdrawn and pipetted into a 50 mL Scott bottle at day 0, day 6, day 12, day 18 and day 24 after storage in oven for further analyses.

Peroxide values (PV)

Peroxide values (PV) of oil samples were according to AOAC Official Method 965.33 (AOAC, 2005).

p-Anisidine values (AV)

p-Anisidine value assay was carried out according to the procedure in AOCS Official Method CD 18-90 (AOCS, 1999).

Total oxidation (TOTOX) values

Total oxidation (TOTOX) values of oil samples were determined using the following equation according to Shahidi and Wanasundara (2008):

$$\text{Total oxidation (TOTOX) value} = 2 \times \text{PV} + \text{AV}$$

Conjugated dienes (CD) and conjugated trienes (CT)

Conjugated diene and conjugated triene of oil samples were assessed based on IUPAC method (1987).

Free fatty acids (FFA)

Free fatty acid content (% FFA) of oil sample was determined according to PORIM (2005).

Iodine values (IV)

Measurement of iodine values (IV) of oil samples were made according to IUPAC (1987).

Total phenolic contents (TPC)

Phenolic compounds were extracted using extraction method according to Abramovicˇ *et al*. (2007) with slight modification. Oil sample (1.75 ± 0.01 g) was weighed, dissolved in 5 mL hexane followed by 10 mL of methanol: water (60:40, v/v) and then centrifuged at 4500 rpm for 5 min. The lower layer, methanolic phase was collected and the upper layer, hexane phase was extracted twice with the previous step to ensure the complete extraction of phenolic compounds. Methanolic phase from the 3 extractions were mixed with 10 mL of hexane to eliminate residual oil samples in a separating funnel. The methanolic fraction was then undergone rotary evaporation at 45°C until dried. It was then reconstituted with methanol: water (60:40, v/v) to dissolve the phenolic compounds with the aids of sonicator (OS-6001 Usonic, OSIM, China).

Statistical analysis

All chemical analyses were performed on duplicate samples and the data were expressed as mean ± standard deviations. Statistical data analysis of various antioxidant assays was conducted using one way analysis of variance (ANOVA) (Minitab Version 13.0) to determine the significant differences ($^{*}P < 0.05$).

Results and discussion

Peroxide values (PV)

The effects of butylated hydroxyanisole (BHA), kenaf seeds extract (KSE), roselle seeds extract (RSE) and roselle extract (RE) added into the sunflower oils, respectively were shown in Table 1. There were regular increases of PVs for all the samples over the storage period. The total increased in PV was followed in descending order by SFO (control) > SFO – BHA > SFO – 1500 KSE > SFO – 1500 RE > SFO – 1500 RSE which achieved a maximum PV of 173.00 ± 14.35, 140.25 ± 11.93, 117.88 ± 1.75, 89.88 ± 2.84 and 94.38 ± 0.63 meq O_2/ kg of oil, respectively at day 24. From the results, it showed that the KSE, RSE and RE controlled PV appreciably at which their PV were lower than BHA at every interval, revealed the antioxidant efficacy in stabilising the oils by delaying the hydroperoxides formation. The maximum range of PV contents of SFO – 1500 KSE, SFO – 1500 RSE and SFO – 1500 RE were far less than those of in the rapeseed oil supplemented with extracts such as sea buckthorn extract and costmary extract which had been previously reported (Bandoniene et al., 2000). These further proved the superiority of KSE, RSE and RE as natural antioxidants.

Table 1. Changes in peroxide values (PV), p-anisidine values (AV) and total oxidation (TOTOX) values of treated sunflower oil samples under accelerated storage*

		Treated sunflower oil samples				
Characteristics	Day	SFO (control)	SFO – BHA	SFO – 1500 KSE	SFO – 1500 RSE	SFO – 1500 RE
Peroxide values (meq O_2 / kg of oil)	0	22.63 ± 2.25^{aA}	20.25 ± 1.71^{aA}	21.38 ± 1.03^{aA}	22.50 ± 2.08^{aA}	20.00 ± 1.47^{aA}
	6	42.13 ± 3.75^{bBC}	40.25 ± 0.65^{bC}	36.88 ± 2.18^{bAB}	34.25 ± 2.18^{bA}	32.25 ± 1.94^{bA}
	12	74.63 ± 5.79^{cD}	67.00 ± 2.48^{cD}	59.50 ± 3.34^{cC}	47.00 ± 1.29^{cA}	54.25 ± 2.18^{cB}
	18	86.00 ± 1.23^{dD}	77.63 ± 2.10^{dC}	70.25 ± 4.43^{dAB}	58.63 ± 1.49^{dA}	67.38 ± 6.65^{dB}
	24	173.00 ±14.35^{eE}	140.25 ± 11.93^{eD}	117.88 ± 1.75^{eC}	94.38 ± 0.63^{eB}	89.88 ± 2.84^{eA}
	Total increased in PV (%)	664.47	592.59	451.36	319.47	349.40
p-Anisidine values	0	2.10 ± 0.17^{aA}	2.14 ± 0.19^{aA}	1.78 ± 0.14^{aA}	2.05 ± 0.21^{aA}	1.92 ± 0.11^{aA}
	6	3.78 ± 0.34^{bB}	3.06 ± 0.14^{bA}	3.66 ± 0.27^{bB}	3.72 ± 0.34^{bB}	3.79 ± 0.06^{bB}
	12	4.63 ± 0.35^{cC}	3.08 ± 0.18^{bA}	3.66 ± 0.18^{bB}	3.80 ± 0.17^{bB}	4.96 ± 0.40^{cC}
	18	6.26 ± 0.53^{dBC}	4.28 ± 0.09^{cA}	5.50 ± 0.42^{cB}	6.33 ± 0.43^{cC}	6.98 ± 0.15^{dD}
	24	13.74 ± 0.49^{eD}	7.96 ± 0.57^{dB}	9.75 ± 1.08^{dC}	7.68 ± 0.52^{dA}	9.54 ± 0.34^{eC}
	Total increased in AV (%)	554.29	271.96	447.75	274.63	396.88
Total oxidation values	0	46.11 ± 4.60^{aA}	41.48 ± 3.19^{aA}	44.45 ± 2.60^{aA}	46.72 ± 5.24^{aA}	40.59 ± 1.48^{aA}
	6	88.03 ± 7.65^{bC}	83.56 ± 1.25^{bC}	77.41 ± 4.40^{bBC}	72.22 ± 4.42^{bAB}	68.29 ± 3.83^{bA}
	12	153.88 ± 11.69^{cC}	135.42 ± 4.67^{cC}	122.66 ± 6.82^{cB}	97.46 ± 3.16^{cA}	113.46 ± 4.75^{cB}
	18	178.26 ± 2.24^{dD}	159.53 ± 4.27^{dC}	146.00 ± 9.12^{dB}	123.33 ± 4.00^{dA}	141.73 ± 13.43^{dAB}
	24	360.21 ± 29.11^{eE}	288.46 ± 24.33^{eD}	245.50 ± 4.51^{eC}	196.43 ± 1.73^{eB}	187.20 ± 4.41^{eA}
	Total increased in Totox value (%)	681.20	595.42	452.31	320.44	361.20

*Values are means ± standard deviation for duplicate analyses of 2 replicates (n=4).
[a] Means in each column followed by different superscripts letters (a-e) are significantly different (*P < 0.05).
[A] Means in each row followed by different superscripts letters (A-E) are significantly different (*P < 0.05).
Abbreviations: PV = Peroxide value, AV = p-Anisidine value, TOTOX = Total oxidation, SFO = Sunflower oil, SFO – BHA = Sunflower oil supplemented with 200 ppm BHA, SFO – 1500 KSE = Sunflower oil supplemented with 1500 ppm kenaf seeds extract, SFO – 1500 RSE = Sunflower oil supplemented with 1500 ppm roselle seeds extract, SFO – 1500 RE = Sunflower oil supplemented with 1500 ppm roselle extract.

p-Anisidine values (AV)

Generally, there were increases of AV for all the samples with irregular patterns (Table 1). Under accelerated storage for 24 days, the total increased of AVs were in the following sequence: SFO (control) > SFO – 1500 KSE > SFO – 1500 RE > SFO – 1500 RSE > SFO – BHA with maximum values of 13.74 ± 0.49, 9.75 ± 1.08, 9.54 ± 0.34, 7.68 ± 0.52 and 7.96 ± 0.57 respectively at day 24. The higher increased of AV in extracts (KSE, RSE and RE) compared to BHA might suggest the reduced capability of extracts in chelating metal ions. Besides, this could be explained that BHA is more effective in retarding the formation of secondary oxidation products (Michotte *et al*., 2011) compared to primary oxidation products. The AV contents of sunflower oils supplemented with extracts in this study were far less than those of in the sunflower and soybean oil supplemented with sesame cake extracts (Mohdaly *et al*., 2010). Therefore, KSE, RSE and RE can be recommended as a potent source of antioxidants for the stabilization of food systems, especially unsaturated vegetable oils.

Total oxidation (TOTOX) values

The TOTOX values of all the samples were increasing in a regular pattern over the storage time. Changes in TOTOX values among the samples were in the following orders: SFO (control) > SFO –BHA

Table 2. Changes in conjugated dienes (CD) and conjugated trienes (CT) content (expressed as extinction values) of treated sunflower oil samples under accelerated storage*

		Treated sunflower oil samples				
Characteristics	Day	SFO (control)	SFO – BHA	SFO – 1500 KSE	SFO – 1500 RSE	SFO – 1500 RE
Conjugated diene values ($\varepsilon^{1\%}_{1cm}\lambda_{233}$)	0	3.48 ± 0.13^{aA}	3.19 ± 0.17^{aA}	3.56 ± 0.24^{aA}	3.16 ± 0.24^{aA}	3.45 ± 0.25^{aA}
	6	6.88 ± 0.13^{bC}	5.64 ± 0.54^{bAB}	5.92 ± 0.08^{bB}	5.46 ± 0.25^{bA}	6.05 ± 0.24^{bB}
	12	11.32 ± 0.27^{cD}	9.26 ± 0.30^{cC}	8.48 ± 0.07^{cB}	7.95 ± 0.39^{cA}	8.65 ± 0.16^{cB}
	18	15.76 ± 0.66^{dD}	11.33 ± 0.13^{dC}	10.64 ± 0.27^{dAB}	10.23 ± 0.41^{dA}	11.08 ± 0.17^{dBC}
	24	25.55 ± 0.38^{eD}	18.26 ± 0.59^{eC}	17.53 ± 0.41^{eC}	13.55 ± 0.33^{eA}	15.13 ± 0.46^{eB}
	Total increased in CD value (%)	634.20	472.41	392.42	328.80	338.55
Conjugated triene values ($\varepsilon^{1\%}_{1cm}\lambda_{268}$)	0	0.76 ± 0.06^{aA}	0.75 ± 0.03^{aA}	0.74 ± 0.03^{aA}	0.74 ± 0.04^{aA}	0.75 ± 0.04^{aA}
	6	1.16 ± 0.02^{bD}	0.96 ± 0.05^{bC}	0.90 ± 0.04^{bBC}	0.81 ± 0.04^{bA}	0.87 ± 0.05^{bAB}
	12	1.65 ± 0.06^{cD}	1.50 ± 0.04^{cC}	1.35 ± 0.06^{cB}	1.28 ± 0.03^{cB}	1.16 ± 0.02^{cA}
	18	2.72 ± 0.07^{dC}	2.36 ± 0.11^{dAB}	2.48 ± 0.12^{dB}	2.32 ± 0.04^{dA}	2.38 ± 0.13^{dAB}
	24	6.21 ± 0.15^{eD}	5.54 ± 0.24^{eC}	5.34 ± 0.16^{eC}	4.65 ± 0.10^{eA}	5.01 ± 0.10^{eB}
	Total increased in CT value (%)	717.11	638.67	621.62	528.38	568.00

*Values are means ± standard deviation for duplicate analyses of 2 replicates (n=4).
[a] Means in each column followed by different superscripts letters (a-e) are significantly different (*P < 0.05).
[A] Means in each row followed by different superscripts letters (A-D) are significantly different (*P < 0.05).
Abbreviations: CD = Conjugated diene, CT = Conjugated triene, SFO = Sunflower oil, SFO – BHA = Sunflower oil supplemented with 200 ppm BHA, SFO – 1500 KSE = Sunflower oil supplemented with 1500 ppm kenaf seeds extract, SFO – 1500 RSE = Sunflower oil supplemented with 1500 ppm roselle seeds extract, SFO – 1500 RE = Sunflower oil supplemented with 1500 ppm roselle extract.

Table 3. Chemical changes, i.e. free fatty acids (FFA) content, iodine values (IV) and total phenolic contents (TPC) of treated sunflower oil samples under accelerated storage*.

		Treated sunflower oil samples				
Characteristics	Day	SFO (control)	SFO – BHA	SFO – 1500 KSE	SFO – 1500 RSE	SFO – 1500 RE
Free fatty acids content (% oleic acid equivalent)	0	0.21 ± 0.02^{aA}	0.20 ± 0.01^{abA}	0.20 ± 0.00^{aA}	0.21 ± 0.01^{aA}	0.20 ± 0.01^{aA}
	6	0.21 ± 0.01^{aB}	0.18 ± 0.01^{aA}	0.21 ± 0.01^{aB}	0.19 ± 0.02^{aAB}	0.19 ± 0.01^{aAB}
	12	0.24 ± 0.02^{abB}	0.20 ± 0.01^{bA}	0.21 ± 0.01^{aA}	0.20 ± 0.01^{aA}	0.20 ± 0.01^{aA}
	18	0.25 ± 0.01^{bBC}	0.21 ± 0.03^{abcA}	0.23 ± 0.02^{aAB}	0.20 ± 0.01^{aA}	0.26 ± 0.01^{bC}
	24	0.36 ± 0.01^{cE}	0.23 ± 0.01^{cA}	0.28 ± 0.01^{bC}	0.26 ± 0.01^{bB}	0.31 ± 0.01^{cD}
	Total increased in FFA (%)	71.43	15.00	40.00	23.81	55.00
Iodine values (g I_2 / 100 g of oil)	0	138.11 ± 0.55^{cA}	137.26 ± 0.55^{dA}	138.00 ± 1.60^{dA}	137.79 ± 1.35^{bA}	138.00 ± 3.81^{bA}
	6	135.57 ± 6.99^{bcA}	132.93 ± 3.88^{cdA}	135.25 ± 3.34^{bcA}	136.63 ± 4.01^{bA}	132.72 ± 2.45^{bA}
	12	134.62 ± 3.90^{bB}	131.13 ± 2.01^{cAB}	131.34 ± 1.74^{bAB}	129.65 ± 0.88^{aA}	131.13 ± 5.93^{abAB}
	18	129.01 ± 0.49^{bB}	126.16 ± 2.05^{bA}	127.43 ± 1.56^{aAB}	128.80 ± 1.27^{aAB}	128.38 ± 2.33^{aAB}
	24	116.04 ± 2.00^{aA}	120.98 ± 0.85^{aB}	125.77 ± 0.24^{aC}	127.61 ± 1.36^{aCD}	126.46 ± 0.24^{aD}
	Total decreased in IV (%)	15.98	11.86	8.86	7.39	8.36
Total phenolic contents (mg GAE / 100 g of oil)	0	11.76 ± 0.40^{eA}	13.84 ± 0.76^{dBC}	13.70 ± 0.45^{dBC}	12.87 ± 0.51^{dB}	13.78 ± 0.25^{dC}
	6	5.11 ± 0.37^{dA}	9.71 ± 0.93^{bB}	4.66 ± 0.26^{dA}	5.17 ± 0.26^{cA}	8.66 ± 0.85^{cB}
	12	3.58 ± 0.37^{cA}	7.24 ± 0.30^{cD}	4.22 ± 0.11^{cB}	4.27 ± 0.28^{bB}	5.45 ± 0.35^{cC}
	18	2.43 ± 0.25^{bA}	7.00 ± 0.16^{cC}	3.11 ± 0.13^{bB}	2.58 ± 0.16^{aA}	2.92 ± 0.10^{bB}
	24	1.53 ± 0.08^{aA}	6.51 ± 0.42^{aD}	2.79 ± 0.14^{aC}	2.53 ± 0.20^{aC}	1.87 ± 0.07^{aB}
	Total lost in TPC (%)	86.99	52.96	79.64	80.34	86.43

*Values are means ± standard deviation for duplicate analyses of 2 replicates (n=4).
[a] Means in each column followed by different superscripts letters (a-d) are significantly different (*P < 0.05).
[A] Means in each row followed by different superscripts letters (A-E) are significantly different (*P < 0.05).
Abbreviations: FFA = Free fatty acid, IV = Iodine value, TPC = Total phenolic content, SFO = Sunflower oil, SFO – BHA = Sunflower oil supplemented with 200 ppm BHA, SFO – 1500 KSE = Sunflower oil supplemented with 1500 ppm kenaf seeds extract, SFO – 1500 RSE = Sunflower oil supplemented with 1500 ppm roselle seeds extract, SFO – 1500 RE = Sunflower oil supplemented with 1500 ppm roselle extract.

> SFO – 1500 KSE > SFO – 1500 RE > SFO – 1500 RSE with maximum values of 360.21 ± 29.11, 288.46 ± 24.33, 245.50 ± 4.51, 187.20 ± 4.41 and 196.43 ± 1.73, respectively at day 24. Table 1 demonstrated the antioxidant activity of BHA in stabilising RBD sunflower oil was not as good as that of in the extracts used in this study, which were KSE, RSE and RE. The low antioxidant activity of BHA in stabilising sunflower oil were mainly due to the less efficacy in delaying hydroperoxides formation as evidenced by high total increase of PV. Besides, it might also due to the fact that BHA is a lipophilic antioxidant which exhibit better antioxidative properties in oil-in-water emulsion rather than in bulk oils (Huber *et al*., 2009).

Conjugated dienes (CD) and conjugated trienes (CT)

The CD and CT values of addition of BHA, KSE, RSE and RE into the RBD sunflower oils during 24 days storage period were shown in Table 2. Generally, the CD and CT values increase regularly with storage period as more hydroperoxides that posses conjugated diene structures were formed and more hydroperoxides degraded into secondary oxidation products with CT structures (Fishwick and Swoboda, 1977). Among the samples, SFO (control) had the highest total increased in CD and CT with 634.20 % and 717.11 % of its initial value, respectively. The high percentages increased in CD and CT over 24 days' storage indicated great intensity of oil oxidation. The CD and CT contents of SFO – BHA, SFO – 1500 KSE, SFO – 1500 RSE and SFO – 1500 RE were also increasing but with a slower rate compared to SFO (control). This demonstrated the antioxidant potential of BHA, KSE, RSE and RE in stabilising oils. On the other hand, SFO – BHA with the higher total CD and CT increase of 472.41 % and 638.67 %, showed incompatible antioxidant properties in preventing lipid oxidation compared to SFO – 1500 KSE, SFO – 1500 RSE and SFO – 1500 RE. Particularly, the CDs of all samples were correlated well with that of in PV as accordance to the finding by Mohdaly et al. (2010). In contrast to the finding reported by Mohdaly *et al*. (2010) which stated that the CT assays showed a good agreement with that of in AV, the CT increase in current study was not correlated well with the AV shown in Table 2. The SFO – 1500 KSE, SFO – 1500 RSE and SFO – 1500 RE showed a higher AV total increased but less CT total increased, relative to SFO - BHA. Since the CT assay is measuring the secondary oxidation products mainly with CT structures, it is not necessary to be corresponded to that of in AV as AV is mainly detecting the aldehydes groups of secondary oxidation products. The presence of other non-conjugated triene aldehydes in SFO – 1500 KSE, SFO – 1500 RSE and SFO – 1500 RE might causes the insignificant readings in CT and AV.

Free fatty acids (FFA)

The FFA contents of SFO (control), SFO-BHA, SFO - 1500 KSE, SFO – 1500 RSE and SFO – 1500 RE during 24 days accelerated storage were shown in Table 3. When comparing among different samples, the total increased of FFA value during 24 days storage were in the order of SFO (Control) > SFO – 1500 RE > SFO – 1500 KSE > SFO – 1500 RSE > SFO – BHA. The higher total increased FFA in extracts (KSE, RSE, RE) compared to that of in SFO – BHA might due to the hydrolytic activity of lipolytic enzymes during the extraction of kenaf seeds, roselle seeds and roselle calyxes. Among extracts, sunflower oil supplemented with RE showed the highest total increased in FFA value with increased of 55.00 % of its initial value. RE exhibited excellent antioxidant activity even comparable to BHA as reported by Mohd-Esa *et al*. (2010). The sudden rose of FFA of SFO – 1500 RE during day 12 to day 18 with increasing rate of 30.00 % was suspicious as RE controlled the FFA well throughout the entire storage period. It might due to the interference by other acids besides FFA such as galacturonic acid that formed upon the hydrolysis of the mucilage of roselle petals (Maganha *et al*., 2010) which lead to sudden increase of it. The FFA of all sunflower oils supplemented with extracts in this study were lower than that of sunflower oils supplemented with carnosic acids that reported by Zhang *et al*. (2010).

Iodine values (IV)

The variations of IV in SFO (control), SFO-BHA, SFO - 1500 KSE, SFO – 1500 RSE and SFO – 1500 RE during 24 days accelerated storage were shown in Table 3. Particularly the IVs of all the samples were decreased over the storage period. As the storage time increases, the free radicals attacked the double bond of the unsaturated fatty acids in sunflower oils resulting in the formation of conjugated bonds (Shahidi and Wanasundara, 1996). Besides, some of the double bonds were destroyed during autoxidation (Zhang *et al*., 2010). During 24 days storage, the total decreased in IV among the samples were in the following order: SFO (control) > SFO – BHA > SFO – 1500 KSE > SFO – 1500 RE > SFO – 1500 RSE. When compared the total decreased of IV in SFO – BHA with that of in extracts (KSE, RSE, RE), SFO – BHA showed higher total decreased of IV compared to that of in extracts (KSE, RSE, RE). The lower IV of SFO – BHA compared to extracts at every interval showed that the lower efficacy of BHA to protect the unsaturated bonds of fatty acids in sunflower oil being oxidised by free radicals. The IV results were also in agreement with the PVs shown in Table 1. The accumulation of hydroperoxides due to the destruction of unsaturated bond by free radicals in BHA was comparably higher than that of in extracts (KSE, RSE, RE).

Total phenolic contents (TPC)

The TPC values of SFO (control), SFO-BHA, SFO - 1500 KSE, SFO – 1500 RSE and SFO – 1500 RE during 24 days accelerated storage were shown in Table 3. Overall, there were decreased of TPC for all the samples throughout the storage period. The lost of antioxidant capability of phenolic compounds during the storage might because of its protective effects against oil deterioration. Besides, along the storage period, there might be thermal degradation of phenolic molecules under extreme temperatures that lead to reduction of TPC (Lafka et al., 2011). The total lost in TPC during 24 days storage were in the following sequences: SFO (control) > SFO – 1500 RE > SFO – 1500 RSE > SFO – 1500 KSE > SFO – BHA. SFO – BHA encompassed the lowest total lost in TPC (52.96 %) with a minimum value of 6.51 ± 0.42 mg GAE / 100 g of oil at day 24. The relatively low total loss of TPC suggested that BHA did not exert their antioxidant activity well in bulk oils. Based on the polar paradox theory by Frankel (1996), it stated that lipophilic antioxidants are more effective in inhibiting oxidation in oil-in-water emulsions, whereas hydrophilic antioxidants are more effective in bulk oil systems. Since BHA is a lipophilic antioxidant, it is dissolved in the oil but not positioned between the oil-air interfaces (Huber *et al*., 2009). As the oxidations of oil were mainly initiated on the oil surface which is in contact with air, BHA definitely unable to provide sufficient protection to the oils preventing it from oxidation by donating hydrogen atoms to free radicals (Laguerre *et al*., 2007). This is in agreement with the relatively high total increase in TOTOX value (595.42 %) shown in Table 1. Besides, the insufficient antioxidant activity of BHA in inhibiting lipid oxidation could also due to the nature of its monohydric phenolic compound which is not as efficacy as polyphenolic compounds present in extracts to prevent lipid oxidation (Daker *et al*., 2008).

The total loss of TPC in SFO – 1500 RSE was relatively low compared to that of in SFO – 1500 RE provided that the SFO – 1500 RSE had higher antioxidant activity as showed by lower total increased in TOTOX value than SFO – 1500 RE. This could be due to the presence of other antioxidants besides polar phenolic compounds such as phytosterols and tocopherols that provide protection against oil oxidation (Mohd-Esa *et al*., 2010). According to

Mohd Nor *et al*. (2008), combination of different antioxidants might provide synergistic effects that further enhanced the oxidative stability of oils. Mohd-Esa *et al*. (2010) reported that the total phenolic concentration was highest in calyxes, followed by leaves and stem. Therefore, the total phenolic contents for the SFO - RE was the highest compared to other extracts for Day 0.

Conclusion

In current study, it could be summarised that the antioxidant activity of kenaf seeds extract (KSE), roselle seeds extract (RSE) and roselle extract (RE) were generally greater than synthetic antioxidant (BHA) owing to their long term effectiveness and steady protection. Among the extracts, RSE showed the highest antioxidant activity followed by RE and KSE. The results were in agreements with Mohd-Esa *et al*. (2010) who reported that roselle seed extracts were found to have the highest antioxidant activity and strongest radical-scavenging activity of all plants tested. Further research on the extraction method is required to improve the quality and bioavailability of extracts.

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References

Abramovicˇ, H., Butinar, B. and Nikolic, V. 2007. Changes occurring in phenolic content, tocopherol composition and oxidative stability of *Camelina sativa* oil during storage. Food Chemistry 104: 903-909.

American Oil Chemists' Society (AOCS), 1999. Official Methods and Recommended Practices of the American Oil Chemists' Society. Campaign: American Oil Chemists' Society.

Association of Official Analytical Chemists. 2005. Official Methods of Analysis. 19th Edition, Washington: AOAC International.

Bandoniene, D., Pukalskas, A., Venskutomis, P.R. and Gruzdiene, D. 2000. Preliminary screening of antioxidants of some plants extracts in rapeseed oil. Food Research International 23 (9): 785–791.

Chan, K.W. and Ismail, M. 2009. Supercritical carbon dioxide fluid extraction of *Hibiscus cannabinus* L. seed oil: A potential solvent-free and high antioxidative edible oil. Food Chemistry 114: 970–975.

Daker, M., Abdullah, N., Vikineswary, S., Goh, P.C. and Kuppusamy, U.R. 2008. Antioxidant from maize and maize fermented by *Marasmiellus* sp. as stabiliser of lipid-rich foods. Food Chemistry 107: 1092-1098.

Fishwick, M.J. and Swoboda, P.A.T. 1977. Measurement of oxidation of polyunsaturated fatty acids by spectrophotometric assay of conjugated derivatives. Journal of the Science of Food and Agriculture 28: 387-391.

Frankel, E.N. 1993. In search of better methods to evaluate natural antioxidants and oxidative stability in food lipids. Trends in Food Science and Technology 4: 220-225.

Ghafar, S.A.A., Yazan, L.S., Tahir, P.M. and Ismail, M. 2010. Kenaf seed super critical fluid extract reduces aberrant crypt foci formation in azoxymethane-induced rats. Experimental and Toxicologic Pathology 1-5.

Halimatul, S.M.N., Amin, I., Mohd.-Esa, N., Nawalyah, A.G. and Siti Muskinah, M. 2007. Protein quality of roselle (*Hibiscus sabdariffa* L.) seeds. ASEAN Food Journal 14 (2): 131-140.

Huber, G.M., Vasantha Rupasinghe, H.P. and Shahidi, F. 2009. Inhibition of oxidation of omega-3 polyunsaturated fatty acids and fish oil by quercetin glycosides. Food Chemistry 117 (2): 290-295.

International Union of Pure and Applied Chemistry. 1987. Standard methods for the analysis of oils, fat and derivatives. 7th Edition, London: Blackwell Scientific.

Krishnaiah, D., Sarbatly, R. and Nithyanandam, R. 2010. A review of the antioxidant potential of medicinal plant species. Food and Bioproducts Processing 157: 1-17.

Lafka, T., Lazou, A.E., Sinanoglou, V.J. and Lazos, E.S. 2011. Phenolic and antioxidant potential of olive oil mill wastes. Food Chemistry 125: 92-98.

Laguerre, M., Lecomte, J. and Villeneuve, P. 2007. Evaluation of the ability of antioxidants to counteract lipid oxidation: Existing methods, new trends and challenges. Progress in Lipid Research 46: 244-282.

Maganha, E.G., Halmenschlager, R.C., Rosa, R.M., Henriques, J.A.P., Ramos, A.L.P. and Saffi, J. 2010. Pharmacological evidences for the extracts and secondary metabolites from plants of the genus Hibiscus. Food Chemistry 118: 1–10.

Michotte, D., Rogez, H., Chirinos, R., Mignolet, E., Campos, D. and Larondelle, Y. 2011. Linseed oil stabilisation with pure natural phenolic compounds. Food Chemistry 129 (3): 1228-1231.

Mohd Nor, F., Mohamed, S., Idris, N.A. and Ismail, R. 2008. Antioxidative properties of *Pandanus amaryllifolius* leaf extracts in accelerated oxidation and deep frying studies. Food Chemistry 110: 319-327.

Mohdaly, A.A.A., Sarhan, M.A., Mahmoud, A., Ramadan, M.F. and Smetanska, I. 2010. Antioxidant efficacy of potato peels and sugar beet pulp extracts in vegetable oils protection. Food Chemistry 123: 1019-1026.

Mohd-Esa, N., Fong, S.H., Ismail, A. and Chew, L.Y. 2010. Antioxidant activity in different parts of roselle (*Hibiscus sabdariffa* L.) extracts and potential exploitation of the seeds. Food Chemistry 122: 1055-1060.

Palm Oil Research Insttitute of Malaysia (PORIM), 1985. Palm Oil Factory Process Handbook Part 1. Kuala Lumpur: Palm Oil Research Institute of Malaysia.

Rozman, T. and Jersèk, B. 2009. Antimicrobial activity

of rosemary extracts (*Rosmarinus officinalis* L.) against different species of Listeria. Acta Agriculturae Solovenica 93 (1): 51–58.

Shahidi, F. and Wanasundara, U.N. 1996. Methods for evaluation of the oxidative stability of lipid-containing foods. Food Science and Technology International 2 (2): 73-81.

Shahidi, F. and Wanasundara U. N. 2008. Methods for measuring oxidative stability in edible oils. In Akoh, C.C. and Min D.B. (Eds). Food Lipids: Chemistry, Nutrition and Biotechnology, p. 387-388. New York: CRC Press.

Sikwese, F.E. and Duodu, K.G. 2007. Antioxidant effect of a crude phenolic extract from sorghum bran in sunflower oil in the presence of ferric ions. Food Chemistry 104: 324-331.

Zhang, Y., Yang, L., Zu, Y., Chen, X., Wang, F. and Liu, F. 2010. Oxidative stability of sunflower oil by carnosic acid compared with synthetic antioxidants during accelerated storage. Food Chemistry 118 (3): 656-662.